Supplemental Tables

Supplemental Table 1: Clinical data for five cases with rearrangements of *PRDM16* at 1p36

	Case 1	Case 2	Case 3	Case 4	Case 5	
Age/sex	48/F	48/F	58/M	61/M	67/F	
Clinical presentation	Anemia.	Lymphadenopathy, splenomegaly.	Splenomegaly, hepatomegaly, lymphadenopathy, fever.	One week history of dyspnea, night sweats, petechial lesions.	Thrombocytopenia, anemia.	Relapse 3 years post-diagnosis.
PB cell counts	WBC 40.5, Hb 5.7, Plt 123, LDH 429	WBC 33.9	WBC 34.2, Hb 9.4, Plt 29, LDH 429	WBC 46, Hb, 7, Plt 7	WBC 4.3, LDH 577	NA
Cytology (PB)	NA	Myeloid blasts (75%), erythroblasts (5%)	Blasts (85%), neutrophils (15%)	Blasts (42%), promonocytes (20%), myelocytes (16%), metamyelocytes (8%), monocytes (3%), neutrophils (4%), lymphocytes (7%)	NA	NA
% Blasts (BM)	80%	90%	NA	49%	26%	NA
Histology (BM)	NA	Hypercellular, megakaryocytes rare, erythroid hyperplasia, mature granulo-monocytes (<10%); MPO+ (20%), Sudan+ (20%), PAS-, Esterases-.	Paratrabecular focal infiltration of B centroblastic centrocytic lymphocytes.	Hypercellular with blast cells (myeloblasts and monoblasts), erythroid component (38%), granulocytes (9%); MPO+ (3%), ANBE+ (15%)	Hypercellular, prominent erythroid hyperplasia, erythroid precursors (50%), dyserythropoiesis; MPO+ (40%) PAS+	NA
Immuno-phenotype	NA	CD34+, CD13+, c-Kit+, CD7+ (major population); CD15+, CD34- (minor population)	CD20+, CD79a+	CD11b+, CD14+, CD34- (monocytic population); CD34+, CD13+, CD15+ (immature population); CD61- (both populations)	CD13+, CD33+, CD34-, GlycoA+, MPO+	NA
Genetics	NA	*FLT3*-D835+	NA	NA	*FLT3* wt, *WT1*+	*NPM* wt
Diagnosis	**AML-M4**	**AML-M1**	**NHL in leukemic phase**	**AML with trilineage dysplasia**	**AML-M6**	**AML-M2**
Outcome	Died after 3 months	Alive	Resistant to conventional chemotherapy	Died 23 days after diagnosis due to respiratory failure and stroke	Remission	Alive

Abbreviations: ANBE, α-naphthyl butyrate esterase; BM, bone marrow; Hb, hemoglobin (g/dL); LDH, lactate dehydrogenase (U/L); MPO, myeloperoxidase; NA, not available; NHL, non-Hodgkin's lymphoma; PAS, periodic acid Schiff; PB, peripheral blood; Plt, platelets ($x10^3/\mu l$); WBC, white blood cells ($x10^3/\mu l$).

Supplemental Table 2: Primer sequences

Experiment	Primer	Sequence 5'-3'	Specificity
5' RACE	RACE4_R (inner)	CTTCTCACTGCCCAGGTCTTCG	Human *PRDM16* ex4
	RACE4_R (outer)	CCAGCCCCCGCCTGATTTGC	Human *PRDM16* ex4
	RACE5_R	CCAGGGGGTAGACGCCTTCCTTC	Human *PRDM16* ex5
	RACE7_R	CTTCCAGTTGAAGGCCTTGG	Human *PRDM16* ex7
RT-PCR of fusion transcripts	BACH2_F	CTCACTGACCTGTCACAAGGTTGCC	Human *BACH2* ex5
	PRDM16_R	CGCATTTGTACTCGCGCTCCTCCGT	Human *PRDM16* ex7
	AML1_F	GAGGGAAAAGCTTCACTCTG	Human *AML1* ex4-5
Sybr® Green Q-PCR	Sybr_F1	CGGCGGCAAAGGAGACAGAC	Human *PRDM16* ex2
	Sybr_R1	ACGCCACACGGATGTACTTG	Human *PRDM16* ex4
	Sybr_F2	CACGAGCACGAGAACGCAC	Human *PRDM16* ex13
	Sybr_R2	GTCCGACTCTGAGGTGGGAG	Human *PRDM16* ex14
	MYB_F	TTGGTCTGTTATTGCCAAGCAC	Human *MYB* ex5
	MYB_R	CTGTCCAGGAGGTTTTCTTAAC	Human *MYB* ex5
	GAPDH_F1	GCCTCAAGATCATCAGCAATGC	Human *GAPDH* ex6
	GAPDH_R1	CCACGATACCAAAGTTGTCATGG	Human *GAPDH* ex7
	PRDM16_gF	GGTCCATGGGAAGGACAGAG	Human *PRDM16* in14
	PRDM16_gR	TCCTGCTTCTCACTGGCTAGG	Human *PRDM16* ex15
	HOX9A_F	AGGAGGCTCATTTGCCCCAG	Human HOX9A in1
	HOX9A_R	CGCATGAAGCCAGTTGGCTG	Human HOX9A ex2
Taqman® Q-PCR	Taqm_F	CGAGGGCGAGGAAGCT	Human *PRDM16* ex1
	Taqm_R	CCCGGTTGGGCTCATACATATTATT	Human *PRDM16* ex1-2
	Taqm_FAM	FAM-CCAAAAGTGACGGTGACGTT	Human *PRDM16* ex2
	Hs00223162_m1	Applied Biosystems	Human *PRDM16* ex14-15
	Hs01922876_u1	Applied Biosystems	Human *GAPDH*
TP53 sequence	TP53_F	ATGGAGGAGCCGCAGTCAG	Human *TP53* ex2
	TP53_R	TCAGTCTGAGTCAGGCCCT	Human *TP53* ex11
	TP53_F_int	AAGACCTGCCCTGTGCAGC	Human *TP53* ex5
	TP53_R_int	ACCTCAGGCGGCTCATAGG	Human *TP53* ex6-7
	TP53_F_ex4	CTGGCCCCTGTCATCTTCTG	Human *TP53* ex4
	TP53_R_ex8	GCACAAACACGCACCTCAAA	Human *TP53* ex8
RT-PCR of mouse tissues	MEL1PR_F *	CTGACGGACGTGGAAGTGTCG	Human *PRDM16* ex3
	MEL1PR_R *	CAGGGGGTAGACGCCTTCCTT	Human *PRDM16* ex5
	MEL1N_F *	CCCCAGATCAGCCAATCTCACCA	Human *PRDM16* ex12
	MEL1N_R *	GGTGCCGGTCCAGGTTGGTC	Human *PRDM16* ex13
	GAPDH_F2	ACCACAGTCCATGCCATCAC	Mouse *GAPDH*
	GAPDH_R2	TCCACCACCCTGTTGCTGTA	Mouse *GAPDH*

* (47)

Supplemental Table 3: Bisulfite sequencing of CpG islands at *PRDM16* putative promoters.

CpG island[A]	Upstream of:	Location (respect to exon)	Product size	# CpGs analyzed	Primers (bisulfite sequencing)[B]
CpG: 129	Exon 1	-6161 to -5878 bp	284 bp	24	**F**: A**TTT**AAAGGGA**TT**TGAGAGGAAAG**TTT**A **R**: **A**CT**A**CCA**AA**A**AA**A**AA**CCCAA**AA**CC
CpG: 406	Exon 1	-1065 to -725 bp	341 bp	44	**F**: **TT**AGAGGGGAGTGT**TTTT**AGTGG**TT** **R**: CCCCACCCAAC**AA**CT**A**CT**A**CTT
CpG: 55	Exon 2	-58 to +245 bp	303 bp	20	**F**: **T**TGTA**T**A**T**A**T**TGGGTGGGG**T**A **R**: A**A**CCCCT**AAA**AT**AA**A**AA**ACTCTC
CpG: 61	Exon 3	-3071 to -2924 bp	148 bp	12	**F**: GGG**TTT**AG**T**TAG**T**AAAATAAAGAGG **R**: CCT**AAA**CA**A**TT**AAA**AACACCAC
CpG: Ex4	Exon 4	-476 to -131 bp	346 bp	13	**F**: GAGTGATGTG**T**AGG**T**TG**TTT**TGAG**T** **R**: CCACCCTCC**AAA**CATCA**A**C**AAAA**CTC

[A] CpG island 406 lies within the promoter of the long isoform, *PRDM16*. CpG islands 129, 55 and 61 are conserved between species and contain potential transcriptional start sites. The CpG cluster upstream of exon 4 is differentially methylated in adult T-cell leukemia (22).

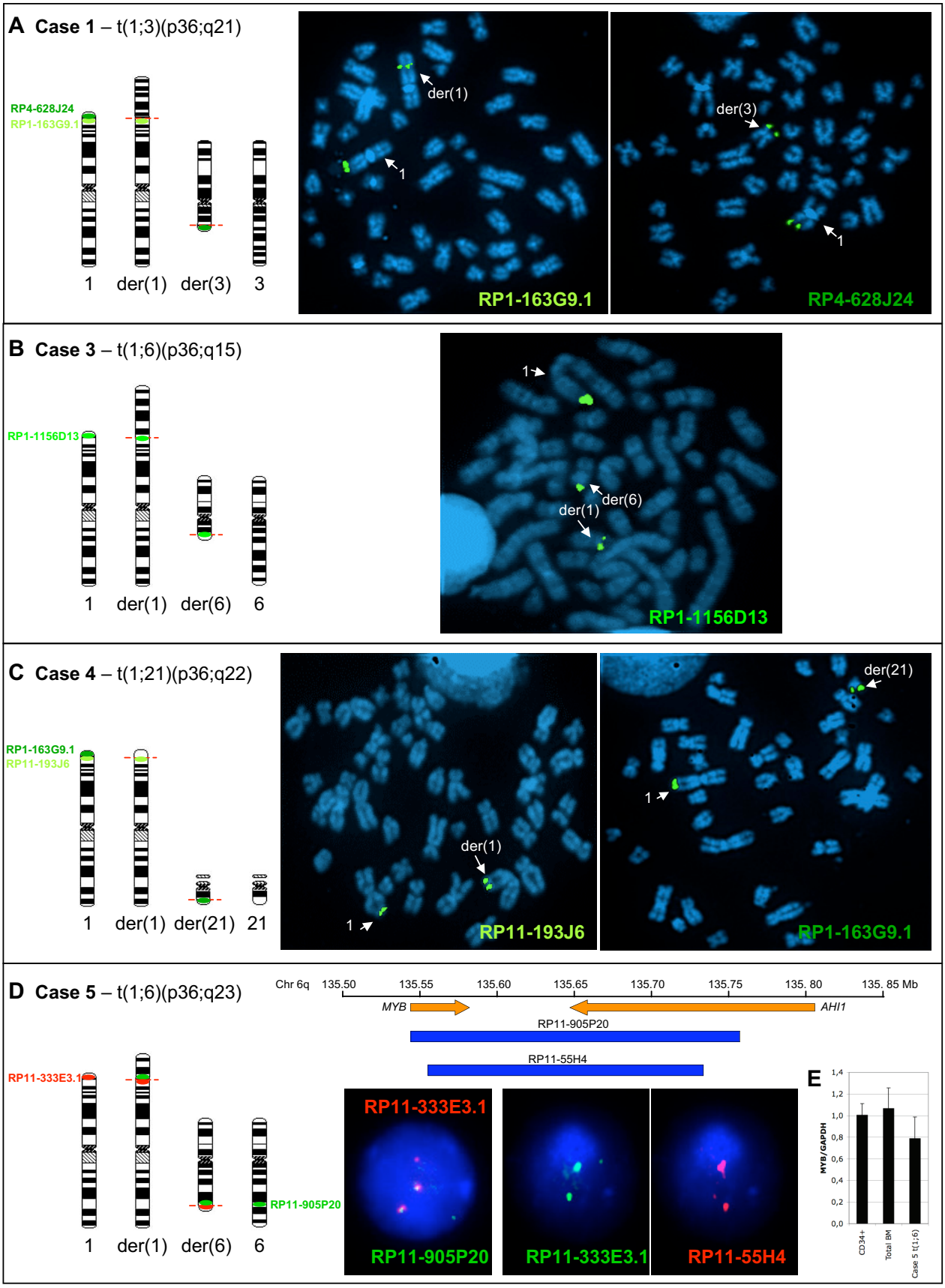
[B] In the forward primer, T represents a C in the normal genomic sequence; in the reverse primer, A represents a G in the complementary strand of the normal genomic sequence.

Supplemental Table 4: Infection and sorting efficiencies

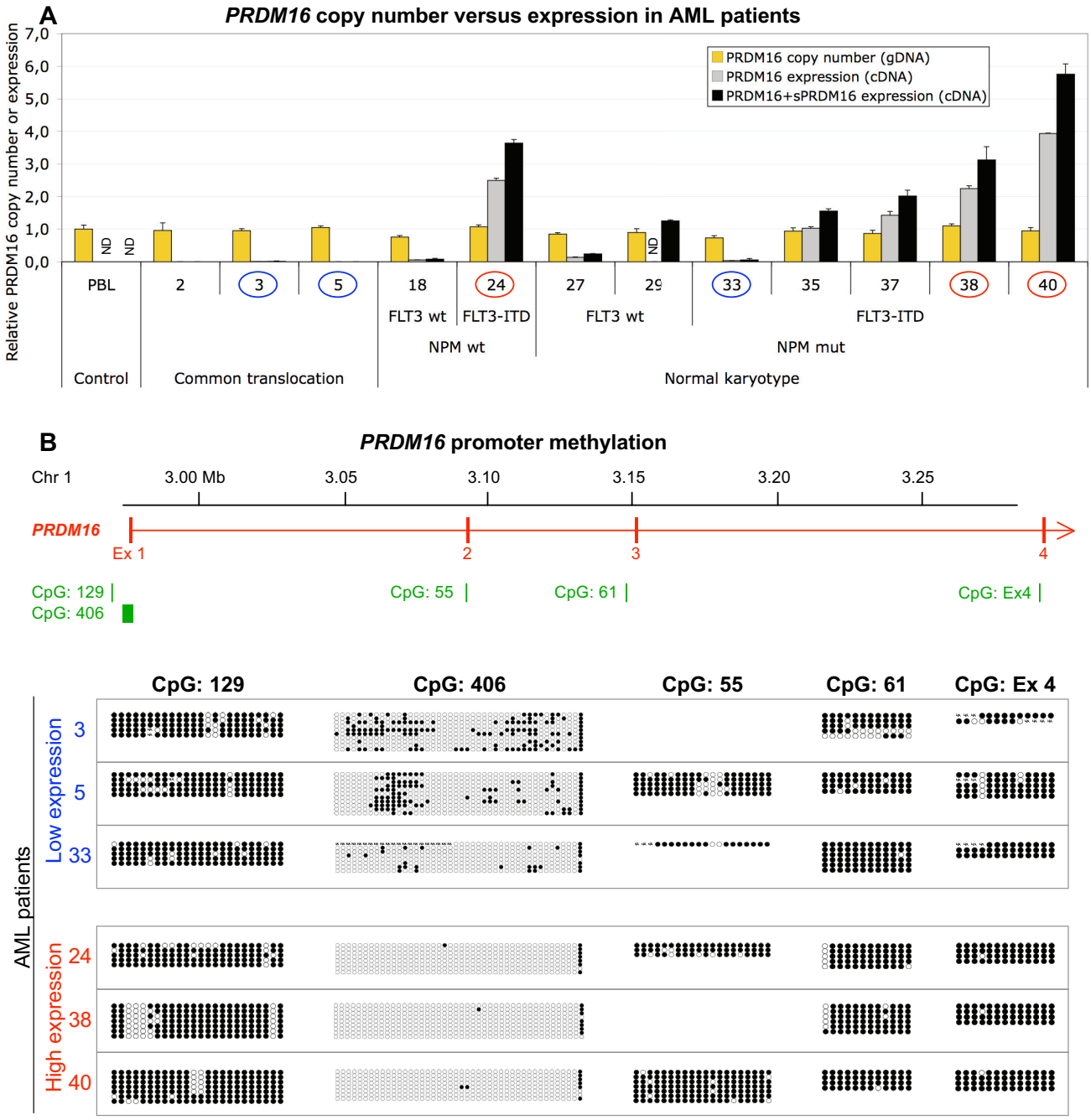
Vector	Lin- cells	% GFP+ cells pre-sorting	% GFP+ cells post-sorting
MSCV	WT	26.0 ± 11.0	91.9 ± 3.2
	p53-/-	30.0 ± 6.6	96.2 ± 1.2
MSCV-PRDM16	WT	10.6 ± 6.3	90.4 ± 1.6
	p53-/-	13.2 ± 3.6	89.2 ± 2.0
MSCV-sPRDM16	WT	14.3 ± 5.4	90.5 ± 2.4
	p53-/-	16.6 ± 4.7	91.5 ± 2.7

Mean ± SD are shown from five independent experiments

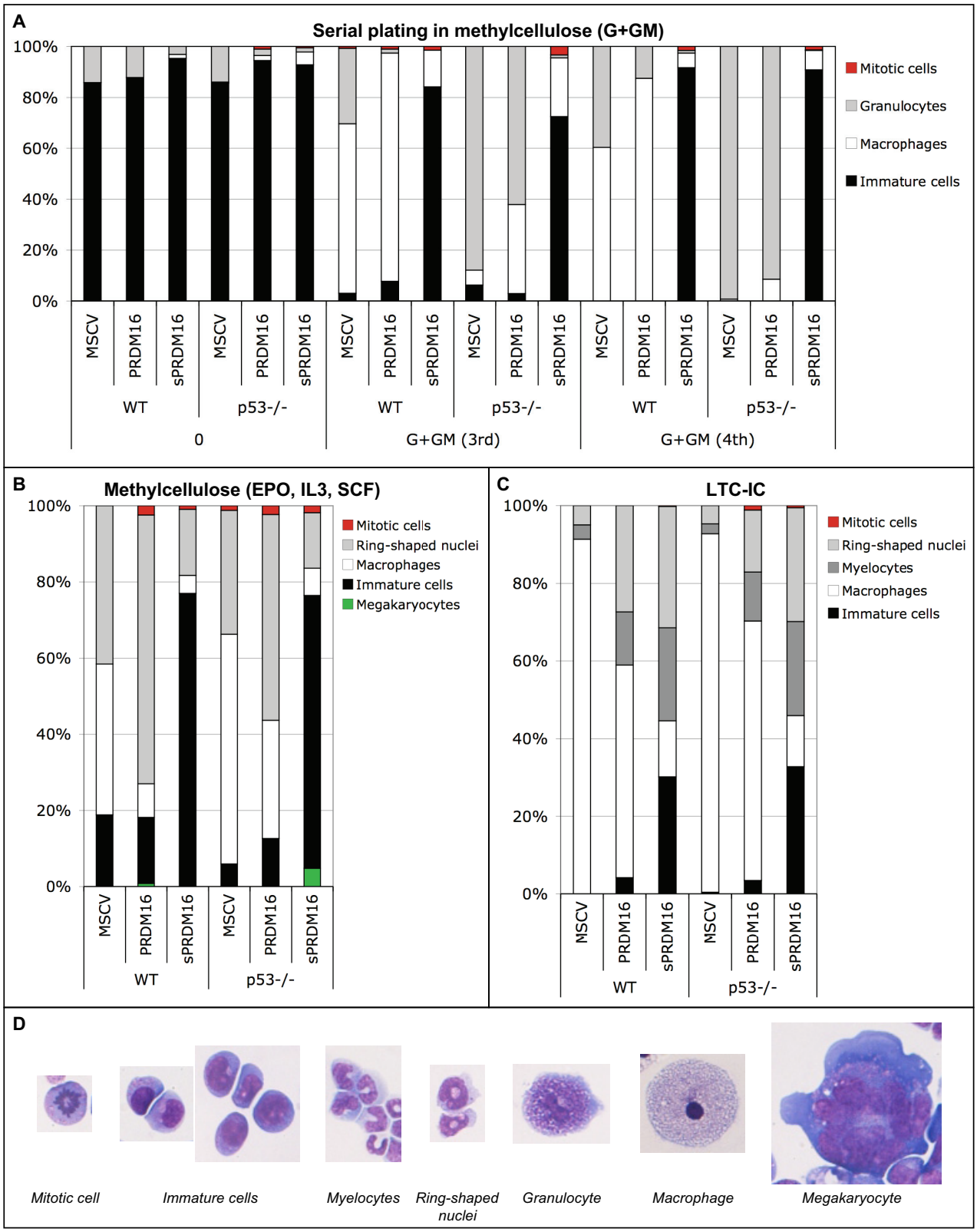
Supplemental Figure 1



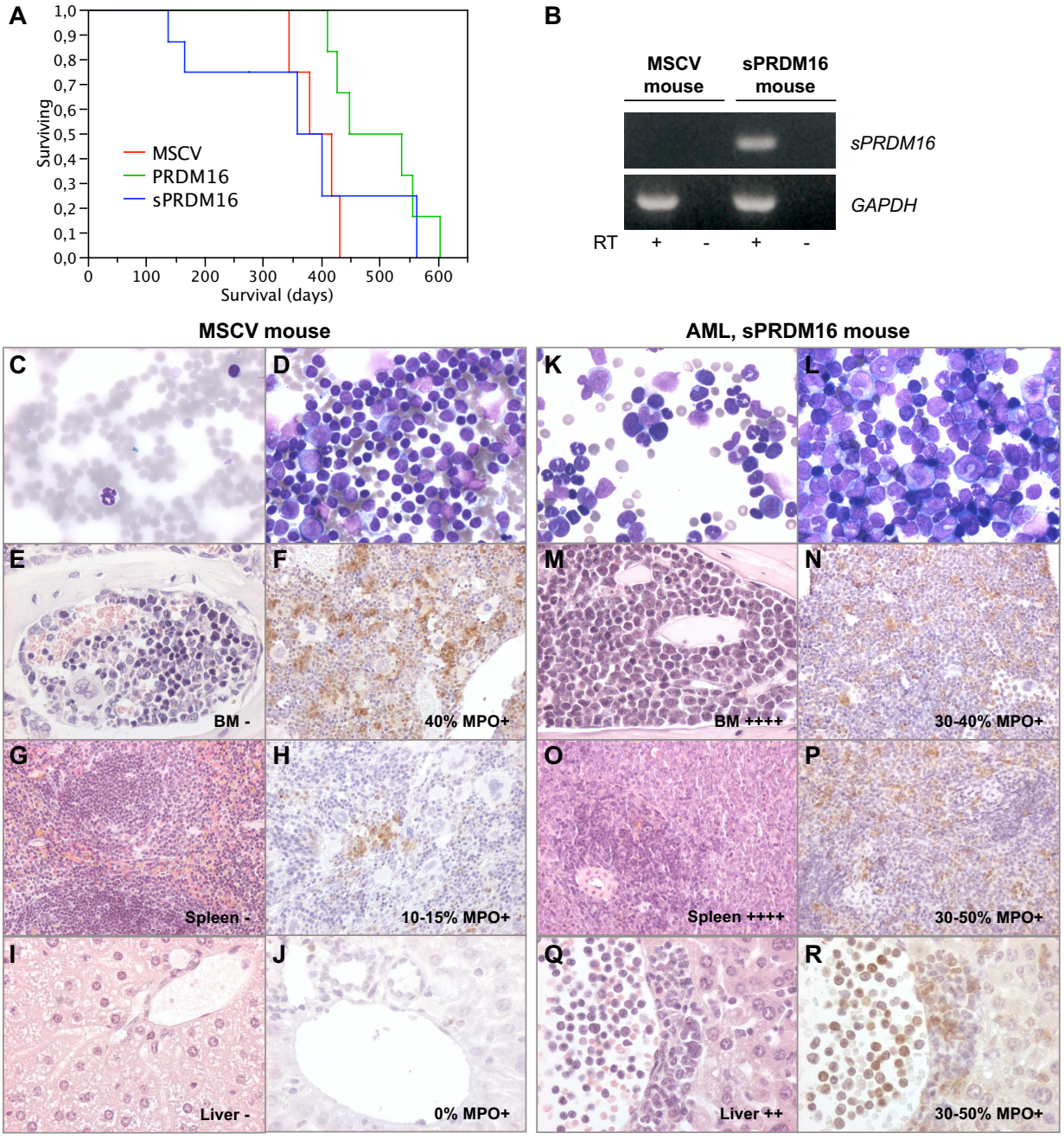
Supplemental Figure 2



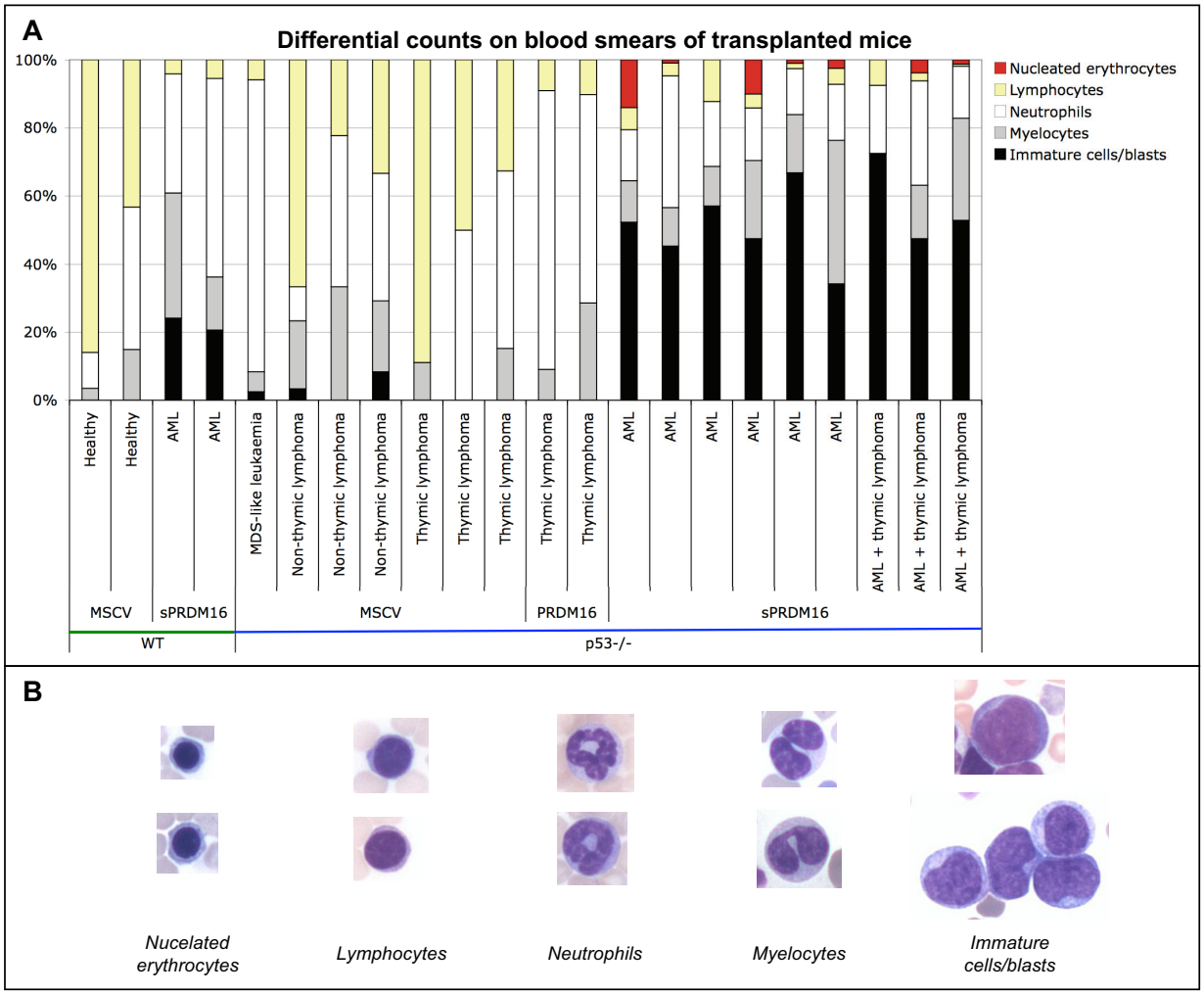
Supplemental Figure 3



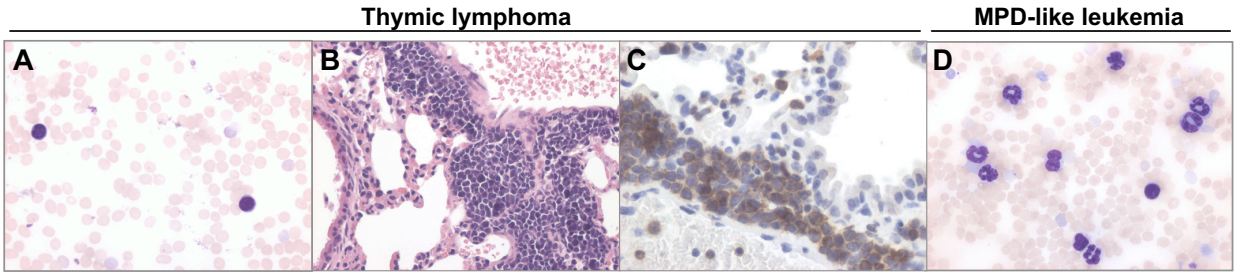
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure Legends

Supplemental Figure 1. FISH mapping of 1p36 breakpoints. A: In Case 1 with a t(1;3)(p36;q21) translocation, the 1p36 breakpoint lies between DNA clones RP1-163G9.1 (left image), hybridizing to derivative chromosome 1, der(1), and clone RP4-628J24 (right image), hybridizing to der(3). A similar pattern of hybridization was observed in Case 2 (not shown). **B**: In Case 3, with a t(1;6)(p36;q15) translocation, the 1p36 breakpoint lies within clone RP1-1156D13, which gave three hybridization signals, one on the normal chromosome 1, one on der(1) and one on der(6). **C**: In Case 4, with a t(1;21)(p36;q22) translocation, the 1p36 breakpoint lies between clones RP11-193J6 (left image), hybridizing to der(1), and RP1-163G9.1 (right image), hybridizing to der(21). **D**: In Case 5, with a t(1;6)(p36;q23) translocation, the 1p36 breakpoint lies within clone RP11-333E3.1 (left and middle images), giving a split signal upon interphase FISH, whilst the 6q23 breakpoint lies within clones RP11-905P20 (left image) and RP11-55H4 (right image), both of which give split signals. **E**: Q-PCR demonstrates that the *MYB* proto-oncogene located at 6q23 in Case 5 is not overexpressed by the translocation. *MYB* expression in Case 5 and in normal total bone marrow (BD Biosciences) was normalized to *GAPDH* and calibrated to levels in normal CD34+ cells.

Supplemental Figure 2. Copy number and CpG island methylation at the *PRDM16* promoter in AML patients. A: *PRDM16* copy number (yellow bars) was determined in genomic DNA derived from 12 AML patients without rearrangements of 1p36. Expression of *PRDM16* (grey) or *PRDM16*+*sPRDM16* (black) is shown for comparison (numbered as in Figure 2C). *PRDM16* copy number was normalized to the copy number of *HOXA9* and calibrated to normal human genomic DNA derived from peripheral blood

lymphocytes (PBL). **B**: Methylation status of the *PRDM16* gene promoter. CpG islands were sequenced for three AML patients with low levels of *PRDM16* expression (patients 3, 5 and 33) and for three patients with high levels of expression (patients 24, 38 and 40). The promoter of the long isoform (CpG: 406) is demethylated in patients showing high levels of *PRDM16* expression. No significant differences are seen at other potential transcriptional start sites located upstream of exon 1 (CpG: 129), exon 2 (CpG: 55), exon 3 (CpG: 61) and exon 4 (CpG: Ex4).

Supplemental Figure 3. Differential counts of lin- cells in in vitro assays. Differential counts (mitotic cells, granulocytes, macrophages and immature cells, myelocytes, ring-shaped nuclei and maegakaryocytes as indicated) of lin- cells transduced with the indicated vectors, before (0) and upon serial replating (third and fourth, as indicated) in the presence of G-CSF and GM-CSF (G+GM, **A**), upon plating in the presence of EPO, IL3 and SCF (**B**) or after long-term culture (**C**). **D**: Representative images of the cell types scored in panels A-C.

Supplemental Figure 4. AML induced by sPRDM16 in a WT background. A: Overall survival of mice transplanted with WT lin- cells transduced with empty vector (MSCV), PRDM16 or sPRDM16. Two of the sPRDM16 mice developed AML at 137 days and 165 days post-transplantation. **B**: RT-PCR evaluation of sPRDM16 expression in the spleen of the leukemic mouse sacrificed at 165 days. Reactions in the presence (+) and absence (-) of reverse transcriptase (RT) are shown. **C-R**: Representative cytological, histological and immunohistochemical analysis from one sacrificed healthy control

MSCV mouse (C-J) and one sPRDM16 leukemic mouse (K-R). **C** and **K**: Peripheral blood (PB) smear (MGG, x400). **D** and **L**: Spleen imprint (MGG, x400). **E** and **M**: Bone marrow (BM) (HE, x400). **F** and **N**: Bone marrow (MPO, x200). **G** and **O**: Spleen (HE, x200). **H** and **P**: Spleen (MPO, x200). **I** and **Q**: Liver (HE, x400). **J** and **R**: Liver (MPO, x400). Neoplastic infiltration for each organ is indicated as: - absent; + minimum (<10%); ++ moderate (10-30%); +++ extensive (30-60%); or ++++ heavy/diffuse (60-100%). The percentage of cells staining positive for MPO also is indicated within the context of the neoplastic infiltrations.

Supplemental Figure 5. Differential counts on blood smears of transplanted mice. **A**: Differential counts (nucleated erythrocytes, lymphocytes, neutrophils, myelocytes, immature cell/blasts) in the peripheral blood of mice reconstituted with WT or p53-/- lin- cells, that were previously transduced with MSCV, PRDM16 or sPRDM16 vectors, as indicated. The health status of the analyzed mice is indicated: healthy, AML, MDS-like leukemia, non-thymic lymphoma, thymic lymphoma. The AMLs caused by expression of sPRDM16 are characterized by the presence of immature cells/blasts in the peripheral blood, comprising 20-25% of nucleated cells in a wild type background and 30-70% of nucleated cells in a p53-/- background. Nucleated erythroid cells are present exclusively in the mice expressing sPRDM16 in a p53-/- background. **B**: Representative images of the cell types scored in panels A.

Supplemental Figure 6. Diagnosis of thymic lymphoma and MPD-like leukemia in a p53-/- background. A: Peripheral blood smear of a PRDM16 (p53-/-) mouse that

developed a thymic lymphoma (MGG, x400). **B-C**: Pathology of a PRDM16 (p53-/-) mouse that developed a thymic lymphoma. Infiltrations comprised 30-60% of the lung (B: HE, x200), of which 90% stained positive for CD3 (C: α-CD3 staining, x400), confirming their T-cell origin. **D**: Peripheral blood smear of the MSCV (p53-/-) mouse that developed an MPD-like myeloid leukemia (MGG, x400). The majority of nucleated cells in the peripheral blood were mature neutrophils, distinguishing this MPD-like myeloid leukemia from the AML observed in the sPRDM16 mice.